2016 FINAL PROJECT REPORT

Microbial Source Tracking And Virginia's Beach Monitoring Program

MEMORANDUM OF AGREEMENT: (VPIMST617GY16)

Between

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Introduction:

Virginia Tech has been conducting quantitative assessment of culturable enterococci and the human-associated microbial source tracking (MST) marker, HF183, on water samples received from Virginia Beaches during the 2016 monitoring season for VDH. Health districts collected and shipped water samples at their discretion when regulatory exceedances of enterococci concentrations occurred. Upon receipt by our laboratory (same day), each sample was analyzed for enterococci using the IDEXX MPN Quanti-tray method to compare to initial water quality data and filtered to collect all biomass and DNA from the water. The filters are then stored frozen for later processing of DNA extraction and quantification of the human-associated HF183 MST markers via quantitative polymerase chain reaction (qPCR) (Seurnick et al., 2005; Griffith et al., 2013). The HF183 marker is recommended as one of the two most sensitive and specific markers of human fecal pollution and is commonly used (Boehm et al., 2013; Griffith et al., 2013). Values for HF183 are reported as copies per volume of water (in this case copies/100mL), which indicates the number of copies of a specific gene sequence from human-associated *Bacteroides* that were detected in each sample. It is important to note that qPCR results have three general outcomes: negative, positive and quantifiable, and positive but not quantifiable (i.e., very low amounts that can be detected but not reliably quantified).

Description of 2016 Advisories:

The VDH-VT Beaches Project began in 2004 when VDH initiated weekly monitoring and implemented EPA-approved sampling protocols. This section is a brief summary of the advisory data compiled by VDH and available at: http://www.vdh.virginia.gov/environmental-epidemiology/beach-monitoring/. The downturn in the number of beaches affected by water quality advisories that was experienced in 2015 did not continue. Water quality in 2016 was more similar to the 2010-2014 period where higher numbers of beaches were affected. In 2016, there were 42 advisories that affected 16 beaches, although the total days under advisory was similar to 2015 (96 in 2016 vs. 97 in 2015). The annual means for 2004-2016 are 24.4 advisories affecting 12.2 beaches for a total of 67.5 days under advisory, making 2016 an above average year. However, it is important to note that 11 of the 15 beaches had three or fewer days under advisory for the entire summer, suggesting only intermittent issues which are to be expected in

summers with heavy rain events such as those that occurred in 2016. For the 42 advisories in 2016, 28 were one day events and 14 were multi-day events.

Table 1. Number of advisories and days under advisory for years 2004 through 2016, all beaches. The number in parentheses indicates number of days under advisory for all beaches with Fairview Beach removed.

Year	# of Advisories	Days under Advisory	# of Beaches
2004	27	147 (122)	11
2005	14	42 (34)	8
2006	8	43 (10)	4
2007	14	50 (18)	8
2008	10	29 (5)	6
2009	14	51 (35)	9
2010	38	81 (63)	16
2011	28	69 (47)	15
2012	23	29 (19)	17
2013	21	30 (25)	13
2014	52	113 (96)	32
2015	26	97 (47)	4
2016	42	96 (61)	16

Fairview Beach (on the Potomac River) was a particularly problematic site, as has historically been the case. In 2016 it had 6 advisories with 35 days under advisory (36% of the 2015 total, *Table 2*). Fairview has not been included in the VDH-VT project in since 2014 and no samples were analyzed by VT from that beach. For that reason, Fairview is not included in the monitoring and source tracking results or discussion in the remainder of this report. As was discussed in previous reports, Fairview was exhaustively sampled and studied by VT and VDH personnel from 2004 through 2012. Multiple sources of input causing the beach contamination were located during the investigations and resolving those inputs will be an expensive and major undertaking. Until such time as those inputs are dealt with, Fairview can be expected to have multiple advisories every summer.

In total there were 8 advisories at Hilton, with 18 days under advisory, including 5 samples with enterococci concentrations above 1,000 MPN/100 mL and one sample above 10,000 MPN/100 mL (*Table 3*). Intensive monitoring at Hilton Beach in previous years demonstrated that high flows generated by stormwater have a direct impact on *Enterococcus* levels at Hilton Beach, as described in previous reports. The open pipe that is present at Hilton

Beach is a storm drain, but water flows out of it in all but the driest weather so it appears that groundwater is infiltrating the storm drain somewhere in the system, which is a common urban infrastructure issue. No dry weather samples were collected in 2016 to test for enterococci, but in the past those samples have been low, suggesting that the *Enterococcus* source is driven by stormwater flow.

Table 2. Number of advisories by beach site for the 2016 season.

Beach	Number of Advisories	Total Days Under Advisory	Days for Each Advisory
15th Street	1	1	1
Fairview	6	35	1, 14, 1, 14, 3, 2
Ocean View Park	1	1	1
Anderson's	4	13	1, 1, 2, 9
North Community	3	3	1, 1, 1
King/Lincoln Park	5	7	1, 2, 1, 1, 2
Huntington	4	4	1, 1, 1, 1
Hilton	8	18	1, 1, 2, 1, 1, 1, 9, 2
East Community	1	2	2
13th View	1	1	1
21st Bay St.	1	2	2
5th Bay St.	1	3	3
10th View	2	2	1, 1
Capeview Ave.	1	1	1
Dam Neck Middle	1	1	1
Captain's Quarters	2	2	1, 1
Totals	42	96	96

Samples collected at the stormwater outfall near Hilton Beach in 2016 again contained significantly higher enterococci concentrations than did the nearby beach water samples (*Table 3*). Although the stormwater outflow was consistently higher, the magnitude of the difference varied widely, ranging from 1.98 times higher on Jul 5 to 42.3 times higher on Jun 1. From these limited data it is reasonable to assume that the stormwater system continues to serve as a source of enterococci to the Hilton beach. However, without coupled hydrologic data that estimate the flow rates, dilution rates, and residence times of the water, it is difficult to say how much of a factor the stormwater outflow was in 2016 compared to other potential nearby or upriver sources.

Aside from Fairview and Hilton, the other three beaches that were moderately impacted during the 2016 season included Anderson, Huntington, and King-Lincoln in the Peninsula

district. Huntington has been the focus of intensive investigation in previous years, with the conclusion that impacts there are primarily downstream effects from contamination at the Hilton Beach site (discussed further in the MST section, below). In 2016, this effect seemed to be smaller than other years, given that Huntington had only 4 total days under advisory (4 advisories of one day each), compared to 18 at Hilton Beach. Problems at Anderson and King-Lincoln were more intermediate, with 4 advisories totaling 13 days at Anderson and 5 advisories totaling 7 days at King-Lincoln.

Table 3. Stormwater outfall impacts on enterococci concentrations at Hilton Beach in 2016.

Date	**enterococci concentration (MPN/100mL)**	
	Hilton Beach	**Hilton stormwater outfall**
5/24/2016	275	581
5/25/2016	*no data*	1,331
5/31/2016	1,619	10,831
6/1/2016	513	22,030
6/2/2016	*no data*	368
6/7/2016	2,602	22,030
6/8/2016	*no data*	752
6/21/2016	738	1,852
6/22/2016	661	*no data*
6/23/2016	12,098	*no data*
6/28/2016	380	1047
6/29/16	153	*no data*
7/5/2016	448	889
7/19/2016	4,639	11,103
8/9/2016	2,351	24,196
8/10/2016	216	747
8/16/2016	*no data*	443
8/23/2016	*no data*	744
9/6/2016	141	480

MST results:

In 2016 the VT lab switched to a quantitative version of the human MST method used in previous years. When quantitative MST is conducted, the results can be interpreted both as the frequency of detection (when multiple samples are collected from the same site) and magnitude of contamination (i.e., higher amounts of the marker indicating higher amounts of contamination or less dilution). This allows not only determination of whether the human marker is present or

absent, as has been reported in previous years, but also gives an estimate of the relative amount of human marker present in each sample. Specifically, the marker targets the DNA sequence from *Bacteroides* cells, which are highly abundant in most mammalian guts. The human-associated HF183 marker targets a particular DNA sequence that has been found to be highly associated specifically with the human gut. The results represent an estimate, obtained from quantitative polymerase chain reaction (qPCR), of the number of copies of the HF183 gene sequence that were present in the sample.

It is important to note that this assay, since it is not reliant on traditionally regulated water quality indicators such as *Enterococcus* and *E. coli*, does not have any established regulatory or public health benchmarks. Ideally, no human MST markers would be present in swimmable waters, but it is currently unclear how much public health risk is associated with presence of the marker at a given concentration. Therefore, these values are best used in a relative manner to track sources in the watershed. In that sense, while the actual copy numbers are reported for all of the quantifiable samples below, they have also been color coded into general categories based upon their relative concentration (*Table 4*). Green boxes are negative samples and of no concern; yellow are detectable but below the limit of quantification (marked as "BQ" = below quantification in the table), which are generally only of relatively mild concern; orange are quantifiable at moderate values (< ~7,000 copies / 100 mL); red are quantifiable at high values (> ~7,000 copies / 100 mL) representing the highest concern. Again, these are not based on any regulatory levels or known epidemiology correlations for the HF183 marker, but only based on relative comparison to values typically seen in environmental samples.

It is also important to note that the quantitative nature of this analysis makes it subject to sample age, since the genetic material decays over time. This issue was identified early in the project, and thanks to cooperation of the health districts, samples obtained later in the project were shipped and analyzed much more quickly than some of the samples early in the summer. As an example, note the differences and amount of decay of measured enterococci in *Table 4* for samples with higher ages compared to those of lower ages. Given that some of the samples were over two weeks old upon receipt, the absolute number of the quantification of HF183 marker from the analysis, particularly for the older samples, was likely to be considerably higher at time of collection. Unfortunately, not enough is known about the decay of the HF183 marker over

time after sample collection to estimate the original concentrations, but the sample age should be considered when interpreting the quantitative data at a specific site.

The HF183 marker was detected in the majority of samples sent to VT in 2016, with 44 of 53 total samples returning a positive result. One sample, from Huntington Beach on May 31, was contaminated in the laboratory and data are not available. However, among the positive samples, 9 were so low as to be unquantifiable, and 4 more were quantifiable but at levels < 1,000 copies/100 mL, making them of relatively low concern compared to more contaminated sites. Samples were sent from 8 beach sites from three districts throughout the summer, but 4 of those sites (3 from Eastern Shore 1 from Hampton) were analyzed only once on Aug 30. All four of these samples had only low to moderate values, and little interpretation is possible from only a single sample at each site. However, if these sites continue to have problems in future years, more MST sampling should be done to more reliably determine potential sources. All other samples came from four beaches – Anderson, Hilton (plus stormwater pipe), Huntington, and King/Lincoln – in the Peninsula district.

Results at both Anderson and King-Lincoln beaches were very similar, with 6 of the 7 samples positive for human contamination at each site. Furthermore, two of the samples at each site – Jun 7and Aug 2 at Anderson Beach, and Jun 7 and Aug 9 at King-Lincoln – were relatively high. This consistent detection of moderate to high amounts of human marker at these sites suggests that additional MST sampling should continue to try determine the location of sources if they experience continued advisories in future years. Of the four samples analyzed for Huntington, only two were positive at moderate levels. Huntington Beach has also been investigated in prior years, with no evidence reported of pollution that originates at Huntington, other than occasional advisories where the *Enterococcus* numbers were just slightly over the standard, which were traced to birds. No local sources of human pollution have ever been reported for Huntington Beach, and previous reports concluded that the primary impact at Huntington is the result of being downstream of Hilton Beach. The low number of samples collected in 2016 make it impossible to determine if that is the case, but it is important to note that on the two dates for which data are available for both Hilton and Huntington – Jun 7 and Jul 19 –concentrations of HF183 were ~2-5 times *higher* at Huntington. This could be for multiple reasons, including analytical variability, local sources of HF183, or sample timing. For example, if the bulk of the contamination occurred the day before at Hilton, it may actually show up as

higher at Huntington on the following day due to transport and dilution. However, with only two samples for comparison it is impossible to determine the cause. If Huntington continues to have problems in future years, additional sampling using quantitative MST (e.g., multi-day following rainfall, longitudinal river transects, etc.) could be used to help differentiate between downstream and local effects at Huntington.

As in previous years, human contamination at Hilton Beach continues to be a problem. Of 14 samples sent from Hilton Beach, 12 were positive, with 9 quantifiable at moderate levels and 1 sample – Jun 23 – quantifiable at a high level. In this regard, it unfortunately appears that the work conducted to date on the nearby sewage system has not drastically reduced or removed the source of enterococci contamination at Hilton Beach. In previous years, reports have concluded that the presence of human marker in the stormwater outflow indicates that leaking sewage infrastructure is potentially contaminating the stormwater system and causing the water quality issues at Hilton Beach. However, the use of quantitative MST in 2016 only partially supports this theory. Clearly, there appears to be some level of human contamination in the stormwater system at Hilton Beach. The amount, though, does not suggest that it is a major contribution to the enterococci detected at the outfall pipe. As seen in *Table 5*, when samples from the same day were compared between the pipe outfall and the beach, the HF183 marker in the stormwater outfall is often equal to or lower than that measured at the beach site. These results suggest that although stormwater outfall might be a significant contributor of enterococci, based on these samples it does not seem to be a consistent contributor of the HF183 marker, which should typically be more diluted at the beach site if this were the case. As discussed for Huntington, however, the *ad hoc* nature of the sampling in the current project is not well suited to making this determination with any certainty. Issues of sample timing could also lead to a misleading interpretation at this site as well. Regardless, these data suggest that more intensive sampling should occur across space and time using quantitative MST, including inflow and outflow of the stormwater system, to more reliably determine if it is serving as a significant source of human marker. If not, efforts to repair the sewage system may continue to fail to address the problem and other animal sources of enterococci in stormwater might have to be examined.

References

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Table 4. Analyzed water quality data showing sample dates and age, enterococci concentrations, and human marker (HF183) results. ENT=enterococci; MPN = most probable number; cp = copies; LOD = limit of detection; BQ = below quantification.

ID	Site	Sample Date	Received-Date	Sample age (days)	Reported ENT (MPN/100mL)	ENT-VT (MPN/100mL)	ENT Percentage Change	Human Marker (cp/100mL)	LOD (cp/100mL)	Report as
Peninsula Health District										
AN1	Anderson Beach	5/31/2016	6/8/2016	8	310	52	-83.2%	0	5882	Negative
AN2	Anderson Beach	6/7/2016	6/23/2016	16	204	10	-95.1%	8,670	526	Positive
AN3	Anderson Beach	8/2/2016	8/5/2016	3	269	201	-25.3%	7,389	500.00	Positive
AN4	Anderson Beach	8/3/2016	8/5/2016	2	369	379	2.7%	1,208	625.00	Positive
AN5	Anderson Beach	8/9/2016	8/12/2016	3	2526	1860	-26.4%	1,538	555.56	Positive
AN6	Anderson Beach	8/10/2016	8/16/2016	6	367	239	-34.9%	1,054	625.00	Positive
AN7	Anderson Beach	8/23/2016	8/26/2016	3	530	266	-49.8%	716	588.24	Positive
H1	Hilton #1 (sw outfall pipe)	5/24/2016	6/8/2016	15	581	63	-89.2%	BQ	5556	Positive, Not quantifiable
H2	Hilton #1 (sw outfall pipe)	5/25/2016	6/8/2016	14	1331	132	-90.1%	BQ	5556	Positive, Not quantifiable
H3	Hilton #1 (sw outfall pipe)	5/31/2016	6/8/2016	8	10831	602	-94.4%	0	6250	Negative
H4	Hilton #1 (sw outfall pipe)	6/1/2016	6/8/2016	7	22030	15531	-29.5%	0	5556	Negative
H5	Hilton #1 (sw outfall pipe)	6/2/2016	6/8/2016	6	368	275	-25.3%	0	5556	Negative
H6	Hilton #1 (sw outfall pipe)	6/7/2016	6/23/2016	16	22030	52	-99.8%	4,790	526	Positive
H7	Hilton #1 (sw outfall pipe)	6/8/2016	6/23/2016	15	752	52	-93.1%	2,170	556	Positive
H8	Hilton #1 (sw outfall pipe)	6/21/2016	7/1/2016	10	1852	148	-92.0%	2,100	526	Positive

H9	Hilton #1 (sw outfall pipe)	6/28/2016	7/1/2016	3	1047	650	-37.9%	2,590	556	Positive
H10	Hilton #1 (sw outfall pipe)	7/5/2016	7/8/2016	3	889	717	-19.3%	1,100	556	Positive
H11	Hilton #1 (sw outfall pipe)	7/19/2016	7/22/2016	3	11103	5794	-47.8%	BQ	625	Positive, Not quantifiable
H12	Hilton #1 (sw outfall pipe)	8/9/2016	8/12/2016	3	24196	14136	-41.6%	2,438	588.24	Positive
H13	Hilton #1 (sw outfall pipe)	8/10/2016	8/16/2016	6	747	364	-51.3%	1,470	500.00	Positive
H14	Hilton #1 (sw outfall pipe)	8/16/2016	8/26/2016	10	443	132	-70.2%	1,104	526.32	Positive
H15	Hilton #1 (sw outfall pipe)	8/23/2016	8/26/2016	3	744	1187	59.5%	1,684	526.32	Positive
H16	Hilton #1 (sw outfall pipe)	9/6/2016	9/9/2016	3	480	160	-66.7%	BQ	526.32	Positive, Not quantifiable
B1	Hilton Beach	5/24/2016	6/8/2016	15	275	51	-81.5%	0	5556	Negative
B2	Hilton Beach	5/31/2016	6/8/2016	8	1619	199	-87.7%	BQ	5263	Positive, Not quantifiable
B3	Hilton Beach	6/1/2016	6/8/2016	7	513	74	-85.6%	0	5556	Negative
B4	Hilton Beach	6/7/2016	6/23/2016	16	2602	216	-91.7%	2,490	556	Positive
B5	Hilton Beach	6/21/2016	7/1/2016	10	738	134	-81.8%	3,970	556	Positive
B6	Hilton Beach	6/22/2016	7/1/2016	9	661	160	-75.8%	2,390	667	Positive
B7	Hilton Beach	6/23/2016	7/1/2016	8	12098	2755	-77.2%	10,200	588	Positive
B8	Hilton Beach	6/28/2016	7/1/2016	3	380	109	-71.3%	1,570	588	Positive
B9	Hilton Beach	6/29/2016	7/1/2016	2	153	146	-4.6%	3,990	556	Positive
B10	Hilton Beach	7/5/2016	7/8/2016	3	488	199	-59.2%	2,040	556	Positive
B11	Hilton Beach	7/19/2016	7/22/2016	3	4639	1467	-68.4%	640	500	Positive
B12	Hilton Beach	8/9/2016	8/12/2016	3	2351	1850	-21.3%	807	625	Positive
B13	Hilton Beach	8/10/2016	8/16/2016	6	216	95	-56.0%	1,619	556	Positive

B14	Hilton Beach	9/6/2016	9/9/2016	3	141	203	44.0%	BQ	556	Positive, Not quantifiable
T1	Huntington Beach	5/24/2016	6/8/2016	15	390	10	-97.4%	0	5556	Negative
T2	Huntington Beach	5/31/2016	6/8/2016	8	1357	156	-88.5%	*no data*	*no data*	*no data*
T3	Huntington Beach	6/7/2016	6/23/2016	16	336	52	-84.5%	5,780	625	Positive
T4	Huntington Beach	7/19/2016	7/22/2016	3	1604	703	-56.2%	3,520	526	Positve
T-5	Huntington Beach	9/6/2016	9/9/2016	3	140	96	-31.4%	BQ	556	Positive, Not quantifiable
KK6	King Lincoln Park	8/23/2016	8/26/2016	3	153	1892	**1136.6%**	1,116	556	Positive
KK1	King-Lincoln Park	5/31/2016	6/8/2016	8	1366	31	-97.7%	0	5556	Negative
KK2	King-Lincoln Park	6/7/2016	6/23/2016	16	5652	160	-97.2%	11,700	526	Positive
KK3	King-Lincoln Park	6/21/2016	7/1/2016	10	133	10	-92.5%	2,060	556	Positive
KK-4	King-Lincoln Park	8/9/2016	8/12/2016	3	27030	19863	-26.5%	7,746	588	Positive
KK-5	King-Lincoln Park	8/10/2016	8/16/2016	6	537	257	-52.1%	BQ	625	Positive, Not quantifiable
KP1	King-Lincoln Pipe	6/8/2016	6/23/2016	15	489	63	-87.1%	1,220	625	Positive
Eastern Shore Health District										
E1	Guard Shore Station 1	8/30/16	9/1/16	2	428	318	-25.7%	2,171	1,250.00	Positive
E2	Guard Shore Station 2	8/30/16	9/1/16	2	118	160	35.6%	BQ	1,250.00	Positive, Not quantifiable
E3	Kiptopeke state Park #1	8/30/16	9/1/16	2	388	1137	**193.0%**	1,893	1,315.79	Positive
Hampton Health District										
BB	Buckroe Beach South Hampton	8/30/16	9/1/16	2	114	63	-44.7%	749	588.24	Positive

Table 5. Stormwater outfall impacts on HF183 concentrations at Hilton Beach in 2016.

Date	**HF183 concentration (CN/100mL)**	
	Hilton Beach	**Hilton stormwater outfall**
5/24/2016	0	BQ
5/25/2016	*no data*	BQ
5/31/2016	BQ	0
6/1/2016	0	0
6/2/2016	*no data*	0
6/7/2016	2,490	4,790
6/8/2016	*no data*	2,170
6/21/2016	3,970	2,100
6/22/2016	2,390	*no data*
6/23/2016	10,200	*no data*
6/28/2016	1,570	2,590
6/29/16	3,990	*no data*
7/5/2016	2,040	1,100
7/19/2016	640	BQ
8/9/2016	807	2,438
8/10/2016	1,619	1,470
8/16/2016	*no data*	1,104
8/23/2016	*no data*	1,684
9/6/2016	BQ	BQ